

Sequence of *MET* protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors

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ABSTRACT We isolated overlapping cDNA clones corresponding to the major *MET* protooncogene transcript. The cDNA nucleotide sequence contained an open reading frame of 1408 amino acids with features characteristic of the tyrosine kinase family of growth factor receptors. These features include a putative 24-amino acid signal peptide and a candidate, hydrophobic, membrane-spanning segment of 23 amino acids, which defines an extracellular domain of 926 amino acids that could serve as a ligand-binding domain. A putative intracellular domain 435 amino acids long shows high homology with the SRC family of tyrosine kinases and within the kinase domain is most homologous with the human insulin receptor (44%) and *v-abl* (41%). Despite these similarities, however, we found no apparent sequence homology to other growth factor receptors in the putative ligand-binding domain. We conclude from these results that the *MET* protooncogene is a cell-surface receptor for an as-yet-unknown ligand.

The *MET* oncogene was isolated from a human osteogenic sarcoma cell line (HOS) treated *in vitro* with the chemical carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (1, 2). Activation of *MET* occurred by way of a DNA rearrangement in MNNG-treated HOS cells (MNNG-HOS cells) that fused sequences from the *TPR* (translocated promoter region) locus on chromosome 1 to sequences from the *MET* locus on chromosome 7 (3, 4). Nucleotide sequence analysis of a portion of the *MET* oncogene (5) showed that it is a member of the tyrosine kinase family of oncogenes (6) and in the kinase domain is most homologous to the human insulin receptor (HIR) (7) and the murine *v-abl* oncogene (8).

The fused *TPR-MET* oncogene expresses a 5.0-kilobase (kb) transcript (3) that encodes a 65-kDa protein that is phosphorylated *in vivo* on tyrosine and serine and *in vitro* only on tyrosine (9) (M. Gonzatti-Haces, personal communication). The activation of the *MET* oncogene by fusion of sequences from chromosome 1 to chromosome 7 resembles the mechanism of activation for several members of the tyrosine kinase family of oncogenes. This includes the rearranged human *BCR-ABL* gene on the Philadelphia chromosome translocation found in chronic myeloid leukemia (10) and the *TRK* oncogene isolated from a human colon carcinoma (11). The *TPR* locus may provide coding sequences to the *MET* oncogene locus or alternatively *MET* activation may be analogous to the *v-erbB* transforming gene of avian myeloblastosis virus that encodes a truncated portion of the epidermal growth factor receptor (EGFR) with an altered kinase activity (12).

The *MET* protooncogene is expressed predominantly as a 9.0-kb RNA species in human fibroblast and epithelial cell lines (3). However, both HOS and MNNG-HOS cell lines express additional *MET*-related RNAs of 6.0 kb and of 7.0 kb.

Three *MET*-related proteins of 110, 140, and 160 kDa can be immunoprecipitated with a *MET* C-terminal anti-peptide antibody from human cell lines expressing the 9.0-kb *MET* RNA (9) (M. Gonzatti-Haces, personal communication). The 140-kDa protein species has tyrosine kinase-specific activity *in vitro* and is phosphorylated on serine and threonine *in vivo*. From these analyses we concluded that *MET* may be similar in function to either membrane growth factor receptors or to nonintegral membrane proteins such as the *abl* and *SRC* members of the tyrosine kinase gene family (6).

In this paper we describe the preparation and nucleotide sequence of cDNA clones covering 7.0 kb of the mRNA expressed by the *MET* protooncogene locus.[§] Using these cDNAs as probes in Southern blot analyses, we estimate that the *MET* genomic locus is at least 100 kb long. The cDNA sequence has a single open reading frame that translates into a protein 1408 amino acids long possessing a structure characteristic of members of the tyrosine kinase growth factor receptor family. However, sequence homology to other growth factor receptor proteins is restricted to the kinase domain.

MATERIALS AND METHODS

Preparation of cDNA Libraries. Two cDNA libraries were constructed with poly(A) RNA isolated from the HOS cell line. An 8- μ g sample was treated with 1 mM methylmercury(II) for 10 min at room temperature and neutralized with 10 mM 2-mercaptoethanol. The cDNA was made according to the method of Gubler and Hoffman (13). Excess linkers (*EcoRI*) were removed when cDNA was size-selected on a Bio-Gel A-50m column. From a total of ≈ 200 ng of cDNA obtained, 40 ng was cloned in the λ gt10 vector to yield $\approx 10^6$ recombinant plaques for each library.

Isolation of RNA and Blot Hybridization. Total cellular RNA was prepared using the guanidinium/cesium chloride method (3). Poly(A)⁺ RNA (5 μ g) was denatured with formamide, fractionated by electrophoresis on 1.2% formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized with 2×10^6 cpm of probe per ml (3).

Nucleotide Sequencing. DNA sequences were determined by the dideoxynucleotide-termination method (14, 15) after subcloning the restriction endonuclease fragments into M13mp18 and mp19 phage vectors.

RESULTS

Isolation and Characterization of *MET* Protooncogene cDNA Clones. To isolate the 5' portion for the *MET* protooncogene 9.0-kb RNA, we constructed two cDNA libraries from HOS cell poly(A) RNA. One library was constructed from

Abbreviations: HOS, human osteogenic sarcoma; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; HIR, human insulin receptor; EGFR, epidermal growth factor receptor.

[§]The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02958).

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oligo(dT)-primed cDNA enriched in double-stranded cDNA molecules >1.5 kb. The second library was primed with a 30-mer oligonucleotide (see Fig. 2) corresponding to *MET* open reading frame in fragment D (Fig. 1A) (3). Both libraries were constructed using the λ gt10 vector system. A third cDNA library prepared from human A431 cell RNA was also screened. Thus, all cDNAs isolated represent portions of the *MET* protooncogene transcripts of 6, 7, or 9 kb in HOS cells or 9 kb in A431 cells and not the *TPR-MET* oncogene RNA of 5.0 kb expressed in MNNG-HOS cells (3).

Initially 200,000 phage from the oligo(dT)-primed cDNA library from HOS cell RNA were screened with a *MET* 3'-region probe (fragment G, Fig. 1A). Seventeen positive plaques were identified, and all phage contained a \approx 1.8-kb *EcoRI* restriction fragment corresponding to the 3' end of the *MET* cDNA (from an internal *EcoRI* restriction site to the linker). The largest cDNA clone (λ met11) contained an additional 2.1-kb *EcoRI* restriction fragment that hybridized with probes further upstream in the *MET* oncogene locus. A cDNA library prepared from human A431 cells was screened with probe G, and 15 positive clones were identified. The largest of these, pmet10, contains a 1.6-kb insert that overlaps with λ met11.

The unamplified oligonucleotide-primed HOS cDNA library was screened using probe D, which contains the primer sequence, and the upstream probe fragment C (Fig. 1A). A total of 30 positive plaques were identified, and two independent recombinants (λ met5 and -14, Fig. 1A) containing the largest cDNA inserts (\approx 4.0 kb) were further characterized. We found that λ met5 and -14 were indistinguishable by restriction endonuclease analyses [e.g., λ met5 and -14 were cleaved by *EcoRI* into three fragments of 2.3, 1.3, and 0.3 kb (Fig. 1A)]. The 1.3-kb fragment of λ met5 and -14 hybridized with fragments D and C of the *TPR-MET* oncogene locus (3) and corresponds to the most 3' portion in λ met5 and -14. All further analyses were performed with the λ met5 insert.

To determine the relationship of the *MET* cDNA sequences to the characterized *MET* RNA transcripts (3), we prepared probes from the 2.3-kb and 1.3-kb subfragments of λ met5, the 1.6-kb fragment of pmet10, and the 2.2-kb and 1.8-kb fragments of λ met11. These were hybridized to RNA prepared from MNNG-HOS, HOS, and CALU-1 cells (Fig. 1B and C). The 1.3-kb fragment of λ met5 (Fig. 1A) plus the pmet10 and λ met11 fragment probes all hybridized with the three *MET* RNA species (9.0, 7.0, and 6.0 kb) in CALU-1, HOS, and MNNG-HOS cells and to the 5.0-kb *TPR-MET* oncogene RNA in MNNG-HOS cells (Fig. 1B). However, a 500-base-pair probe derived from a 5' subfragment of the 2.3-kb *EcoRI* fragment of λ met5 hybridized only to the 9.0-kb *MET* protooncogene transcript (Fig. 1C).

The restriction map of the overlapping cDNA clones as represented in the 9.0-kb *MET* protooncogene RNA is depicted in Fig. 1A. We have not excluded the possibility that the 7.0 kb of overlapping cDNA does not fully represent the 9.0-kb *MET* transcript. However, in heteroduplex analysis the size and complexity of the human cDNA clones compare favorably to a 6.8-kb mouse *met* cDNA (A. Iyer, unpublished results). Furthermore, we have isolated overlapping cDNA from several independent libraries and find no evidence from restriction analysis for heterogeneity. The 9.0-kb RNA size is most likely an overestimate from gel electrophoretic mobility measurement of the size of the actual RNA transcript (3).

Nucleotide Sequence of *MET* cDNA and the Deduced Protein Sequence. The sequence of the overlapping cDNA clones corresponding to the *MET* protooncogene 9.0-kb transcript reveals a single open reading frame of 4224 nucleotides (Fig. 2). The first ATG codon found in this open reading frame at nucleotides 1–3 and its context match the consensus sequence for a translation initiation site (16). We assume that this is the initiation site for translation, since additional ATGs preceding this codon are immediately followed by termination codons in all three reading frames. We also find multiple termination codons downstream of the open reading frame that ends at nucleotide 4225. The 3' 1.8-kb *EcoRI* restriction fragment of the λ met11 clone (Fig. 1A) contains the 3'-noncoding sequence, poly(A) adenylation signal, and a portion of the poly(A)⁺ tract (data not shown).

The predicted primary structure of the *MET* protooncogene protein product is similar to other cell-surface growth factor receptors (Fig. 2). The first 24 N-terminal amino acids with the exception of the arginine (residue 21) are hydrophobic and could serve as a signal sequence for transporting the protein into the lumen of the endoplasmic reticulum. By analogy to the EGFR or HIR this sequence may be cleaved after glycine-24 in *MET* since signal peptidases frequently cleave after this amino acid residue (17). Hydropathy analysis also reveals a stretch of 23 amino acids (residues 950–973) that are strongly hydrophobic and could serve as a transmembrane domain for the putative receptor (Fig. 2). This sequence like other transmembrane domains is followed immediately by a cluster of hydrophilic amino acids (Lys-Lys-Arg-Lys, residues 974–977) that can serve as a cytoplasmic anchor. This delineates residues 24–950 as a putative extracellular domain that is analogous to the ligand-binding domain of other receptors.

Residues 973–1408 constitute the cytoplasmic intracellular domain and contains the phosphotransferase effector moiety. The sequence of this region contains a consensus ATP-binding domain and a kinase domain (residues 1101–1351) that is highly homologous with the SRC family of tyrosine kinases (6) where the closest match is with the HIR (44%) (7),

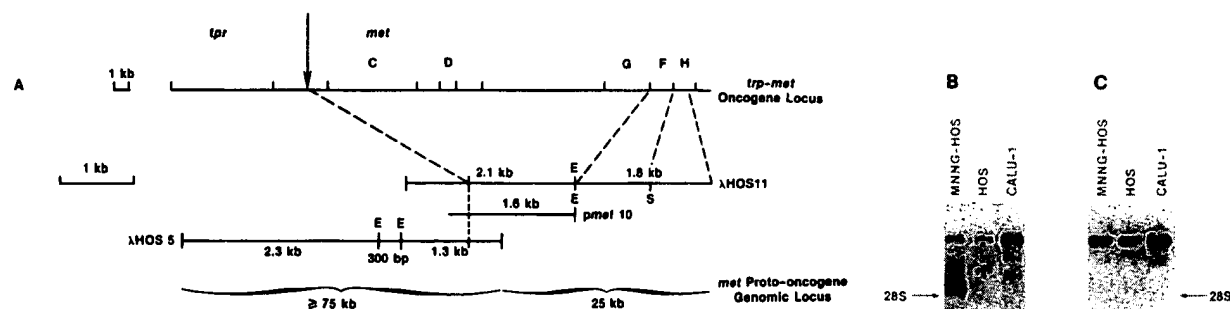


FIG. 1. (A) Schematic diagram showing the *TPR-MET* oncogene locus and the probes used for analysis of cDNA clones. This diagram is a composite of the three overlapping cDNA clones λ met11 (λ HOS11), pmet10, and λ met5 (λ HOS5). The *EcoRI* restriction endonuclease sites (E) for each cDNA clone are marked. (B and C) RNA gel blot hybridization analysis of human and *MET* NIH 3T3-cell transformant mRNAs. Poly(A)-selected RNA (5 μ g) from HOS, MNNG-HOS, and *MET* NIH 3T3 transformant 2212C (CALU-1) cells was hybridized with radiolabeled *MET* protooncogene cDNA fragments, a 1.3-kb *EcoRI* subfragment from λ met5 (B) and a 500-base-pair *EcoRI-Pst* I subfragment of λ met5 (C).

FIG. 2. *MET* protooncogene cDNA clones, nucleotide sequence, and deduced amino acid sequence. Nucleotides are numbered above and amino acids are numbered below the line starting with the first methionine of the open reading frame. The putative signal sequence and transmembrane domain are underlined with a solid bar. The consensus sequences for N-linked glycosylation are underlined, and the oligonucleotide primer is shown as 1. Cysteine residues are boxed. Putative ATP binding domain: *, lysine; ***, Gly-Xaa-Gly-Xaa-Xaa-Gly. Sequence analysis was performed using the ANALSEQ and IDEAS program packages of R. Staden (Medical Research Council, U.K.) and M. Kanehisa (Kyoto University, Japan), respectively. Alignments of protein sequences were performed with the ALIGN program of B. Orcutt, M. O. Dayhoff, D. G. George, and W. C. Barker (National Biomedical Research Foundation, Washington, DC).

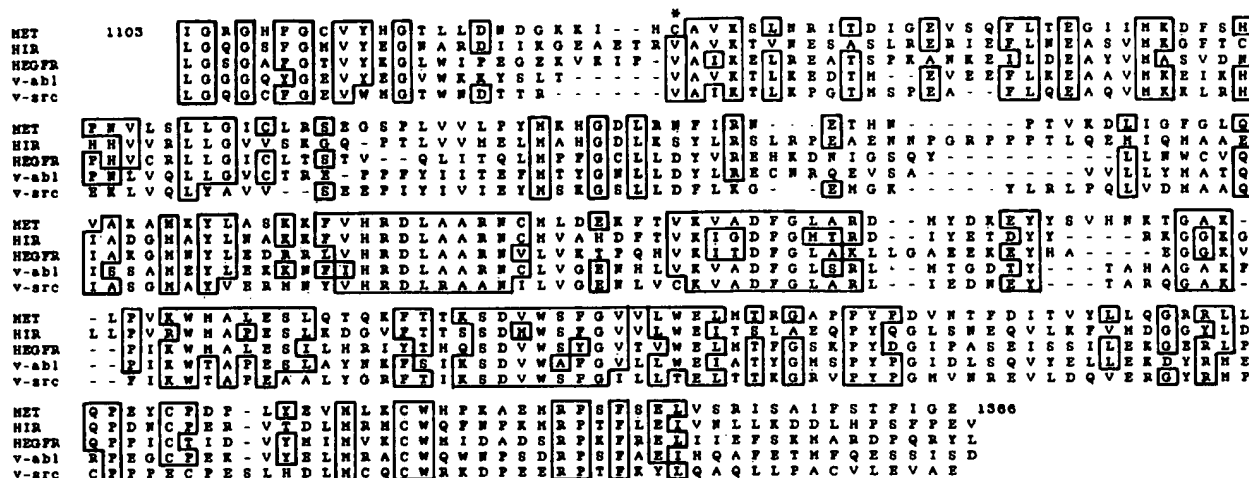


FIG. 3. Comparison of the putative tyrosine kinase domain of the *MET* gene product (residues 1103–1366) with the corresponding domains of HIR, human EGFR (HEGFR), and the products of the *v-abl* and *v-src* genes. All residues shared between *MET* and two or more of the other kinases are boxed. The aligned sequences show homologies of 44% with HIR, 38% with HEGFR, 41% with *v-abl*, and 37% with *v-src*. Hyphens are gaps inserted to maximize the number of matches in the comparison.

the murine *v-abl* gene (41%) (8), and the human EGFR (38%) (12) (Fig. 3). There are two tyrosine residues (positions 1252 and 1253) in the *MET* kinase domain that could correspond to tyrosine-416, the major site of autophosphorylation in pp60^{v-src} (6). However, it is not yet known which tyrosine residue is phosphorylated in the *MET* oncogene or proto-oncogene product. A putative ATP-binding site at Lys-1127 is 20 amino acids downstream from a consensus Gly-Xaa-Gly-Xaa-Gly (6) (residues 1102–1107). When compared with other kinases, the ATP-binding domain in *MET* is further from the transmembrane domain (130 amino acids), for example, only 49 amino acids separate the ATP-binding domain from the transmembrane domain in the EGFR/transforming growth factor type α receptor (12) or HIR (7) (Fig. 4). Moreover, unlike other known tyrosine kinases, *MET* contains a cysteine in the ATP-binding domain (residue 1126) instead of a valine residue.

The breakpoint of the DNA rearrangement that activates the *TPR-MET* oncogene (3) is located at nucleotides 3081/3082 in the *MET* protooncogene sequence (Fig. 2). This breakpoint is located 54 amino acids from the transmembrane domain; thus, the *TPR-MET* oncogene contains essentially only the kinase domain from the *MET* protooncogene and not the transmembrane domain. This correlates with protein data that shows that the *TPR-MET* oncogene protein product is not surface iodinated, is located in soluble cytoplasmic cell fractions, and has kinase activity (M. Gonzatti-Haces, personal communication). The structure of *TPR-MET* is different from that of the *v-erbB* or *v-ros* (18), oncogenes that contain transmembrane domains and have lost only the extracellular ligand-binding domain, although we expect from other studies (M. Gonzatti-Haces, personal communication) that *TPR* contributes coding sequences.

DISCUSSION

In this paper we predict the protein structural outlines of the *MET* protooncogene product based on nucleotide sequence of cDNA. We isolated overlapping cDNA clones that correspond to the 9.0-kb *MET* transcript expressed in HOS cells (3). The complete cDNA sequence has an open reading frame of 4224 nucleotides that could code for a protein of 157 kDa. This is consistent with the size of the *MET* protein products (110, 140, and 160 kDa) that have been detected with a C-terminal peptide antiserum (ref. 9 and M. Gonzatti-Haces, personal communication). The primary amino acid sequence

shows structural features characteristic of the growth factor receptor tyrosine kinase gene family (Fig. 4).

We had noted that sequence comparisons have revealed homologies between the primary structures of receptors. To determine if *MET* was at all homologous to known receptor or receptor-like proteins, we have searched the National Biomedical Research Foundation protein data bank[†] and directly compared the *MET* protooncogene with the EGFR (12, 19), HIR (7), human insulin growth factor type 1 receptor (20), colony-stimulating factor type 1 receptor (21), platelet-derived growth factor receptor (22), low density lipoprotein receptor (23), transferrin receptor (24), and the epidermal growth factor precursor (25) using the ALIGN program of Dayhoff *et al.* (26). This direct comparison revealed no significant homology with any of these proteins outside of the kinase domain. A comparison of the structural features of the *MET* kinase domain with *v-fms*/colony-stimulating factor type 1 receptor (21), platelet-derived growth factor receptor (22), or *v-kit* (27), which have a split kinase domain (Fig. 4), showed that the *MET* kinase is not interrupted (Figs. 3 and 4). This suggests that *MET* should be included in the kinase subgroup that includes the EGFR/transforming growth factor type α (12, 19), HIR (7), human insulin growth factor type 1 receptor (20), and *v-ros* (18) that have a single kinase domain.

In comparison with other receptors, *MET* contains a large putative extracellular domain with a characteristic array of cysteine residues (Fig. 4). Cysteine-rich clusters are thought to form an essential structural backbone for the ligand-binding domain. In contrast to the platelet-derived growth factor receptor or colony-stimulating factor type 1 receptor, which have cysteine residues spaced throughout the extracellular domain, the EGFR/transforming growth factor type α receptor or HIR contain cysteine-rich domains (Fig. 4). Although the putative extracellular domain of *MET* contains one small cysteine-rich domain (11 out of 105 amino acids, residues 520–625; Figs. 2 and 4), the majority of the cysteine residues (22 out of 34) are dispersed. The *MET* protein, therefore, appears to contain cysteine density features in common with both classes of receptors.

Although three *MET*-related polypeptides of 110, 140, and 160 kDa have been observed in human cell lines only the 140-kDa *MET* is labeled when cells are surface iodinated and is,

[†]Protein Identification Resource (1986) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 10.

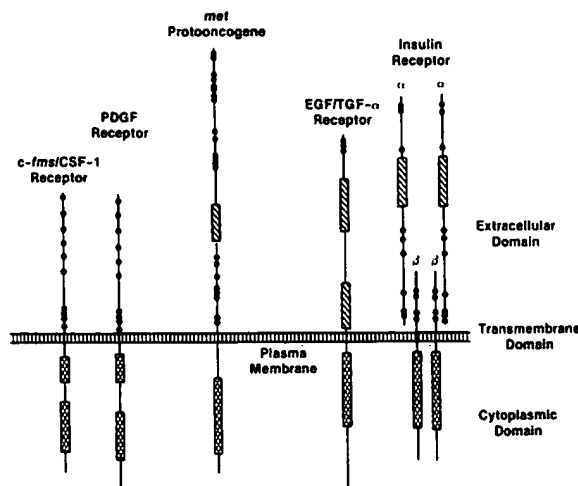


FIG. 4. Schematic comparison of *MET* protooncogene with other cell-surface receptors. Cysteine-rich domains are shown in hatched boxes; other cysteine residues in the extracellular domain are represented as solid circles. Tyrosine kinase domains are cross-hatched boxes. CSF-1, colony-stimulating factor type 1; PDGF, platelet-derived growth factor; TGF- α , transforming growth factor α .

therefore, present on the cell surface (ref. 9 and M. Gonzatti-Haces, personal communication). The 140-kDa *MET* product could correspond in size to the protein predicted from the *MET* open reading frame (157 kDa), suggesting that it is not extensively posttranslationally modified. The putative extracellular domain of *MET* contains 11 potential sites for N-linked glycosylation (Fig. 2), but the 110-, 140-, or 160-kDa products do not appear to be heavily glycosylated (M. Gonzatti-Haces, personal communication). Analysis of the predicted *MET* protein secondary structure using the program of Stephens (28) with the algorithm of Hopp and Woods (29) showed that none of the potential N-linked glycosylation sites in *MET* occur in strongly predicted β -turn structures. Since studies have shown that $\approx 67\%$ of N-linked glycosylation sites of proteins occur at β -turn structures (30) few of the potential sites in *MET* may be available for glycosylation.

These data are consistent with the proposal that the *MET* protooncogene represents a transmembrane protein that may act as a receptor for an as-yet-unknown ligand. Given that phosphorylation of tyrosine and serine/threonine may play important physiological roles for several growth factor receptors and protooncogenes, the fact that the *MET* protooncogene is expressed predominantly in human fibroblast and epithelial cell lines and rarely in cell lines of hematopoietic lineage (3) indicates that *MET* may be a cell-lineage-specific receptor for a growth or differentiation factor.

The *MET* oncogene locus was shown to be closely linked to the inheritance of the recessive genetic disorder cystic fibrosis (31) thus localizing the cystic fibrosis locus to human chromosome 7q21-31 (5, 31). Using *MET* cDNA we have identified a new restriction fragment length polymorphism (RFLP) (32) ≈ 100 kb from *MET* oncogene probes originally used for RFLP analysis and prenatal diagnosis. This RFLP will increase the confidence limits with which these analyses can be performed.

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